The role of selenium content of wheat in the human nutrition. A literature review

M. Tamás¹
email: tamasmelinda@sapientia.siculorum.ro

Zs. Mándoki²
email: mandoki.zsolt@ke.hu

J. Csapó¹,²
email: csapo.janos@ke.hu

¹Sapientia–Hungarian University of Transylvania, Csíkszereda Campus, RO-530104, Libertății 1., Miercurea-Ciuc
²University of Kaposvár, Faculty of Animal Science, Guba S. u. 40, 7400 Kaposvár, Hungary

Abstract. The authors discuss the importance of the selenium content of wheat in the human nutrition during which they deal with selenium as a component of enzymes, selenium deficiency of domestic animals, the role of selenium in the nutrition, in this connection the consequences of selenium deficiency and toxicity of selenium. Thereafter, analyzing the selenium content of wheat and its utilization, the contribution of cereals to the human’s selenium demand, selenium content of wheat cultivated on different soils, effect of technology (grinding) on the selenium content of flour, selenium species occurring in wheat, bioavailability of selenium content of various foods as well as the effect of selenium on the enzyme activity in wheat are treated. In the second part of the study availability of selenium content of soil is analyzed in different plants, mainly in

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wheat, possibilities for increasing selenium content in foods by increasing selenium content of soil is investigated, and finally incorporation of selenium in wheat is treated. It is established that selenium is an essential element for the human organism as it is co-factor of many enzymes. It also discussed that certain parts of the world can be extremely selenium deficient, and there are also such areas where selenium content of the soil reaches a toxic level. Different selenium species are utilized differently in the humans and in animals, therefore it is not enough to analyze only the total selenium content but also the selenium species should be analyzed. Finally, it is established that by increasing the selenium content of soil the selenium content of wheat can be considerably increased, which as being the most important cereals, can considerably contribute to the satisfaction of the human selenium requirements.

1 The role of selenium in the human nutrition

Selenium as enzyme component

Selenium can be present in foods as essential nutrient or toxic material, as selenium known earlier merely as carcinogenic and toxic material (Whanger, 2002) found even if in small amount to be essential for animals at the end of the 1950’s (Schwarz and Foltz, 1957), since selenium is an essential component of more than 30 selenoproteins and selenoenzymes, in mammals (Brown and Arthur, 2001; Rayman, 2002). Properties and biological functions of around 15 selenoenzymes including the antioxidant glutathione-peroxidases (GPx) were discovered, three forms of thioredoxin reductases playing important role in regeneration of the antioxidant system of the organism and contributing to the establishment of the intracellular redox status.

The properties of three iodothyronine deiodinase enzymes that are playing a role in the formation of the thyroid hormone were also described (Brown and Arthur, 2001; FAO, WHO, 2001; Rayman, 2002). In these selenoproteins selenium is present in the form of selenocysteine (Se-Cys) determined by the codon UGA, typically a stop codon, during the ribosomal protein synthesis (Low and Berry, 1996; Stadtman, 1996). Under normal physiological conditions selenium occurs in Se-Cys almost fully ionized which provide an especially effective biological catalysis for the selenoproteins (Brown and Arthur, 2001; Stadtman, 1996). In plants, selenium has no known functional effect. Incorporation of the selenoamino acids into the plants happens by replacement of cysteine and methionine which is usually associated with harmful consequences for the plants.
Selenium deficiency of domestic animals

Selenium deficiency of domestic animals is well-known, white-muscle disease of calves and sheeps is induced by selenium deficiency. Based on the literature it appears that out of the domestic animals the sheep, mainly the young lamb is the most sensitive to the selenium deficiency, but it is very important to satisfy the selenium need of all the other animals as well. Serdaru et al. (2003) examined the selenium status of 185 feeding stuff samples (hays, green plants and feedstuff concentrates) cultivated in south-west Romania (Dobrudja). Selenium content of the samples was determined spectrofluorometrically after derivatization with 2,3-diaminonaphthalene. Only 6.5% of the samples contained an appropriate selenium content (0.15-0.30 mg/kg), while 93.5% of the analyzed samples had a selenium content ranging 0.001-0.150 mg/kg that is, these feedstuffs were selenium deficient. Based on the selenium content the samples were divided into three groups: 3.2% very deficient (selenium content below 0.01 mg/kg), 84.9% critical (selenium content between 0.01-0.1 mg/kg), and 5.4% borderline case with a selenium content of 0.1-0.15 mg/kg. Summarized, it can be said that the feeding stuffs cultivated in Dobrudja are in general selenium deficient and no question about that actions should be taken in order to eliminate selenium deficiency of animals.

Vignola et al. (2009) examined and evaluated the performance, the quality and oxidative stability of meat, the total Se and specific selenoamino-acids content of muscle of lambs that were fed diets supplemented from different Se sources and at different levels. Forty-eight Apennine lambs 30 day old (12.78 ± 0.94 kg) received, during a 63 day period, a total mixed ration (TMR) which was either Se unsupplemented (Control group – background only – 0.13 mg/kg Se) or supplemented with Na selenite (0.30 mg/kg Se as sodium selenite) or selenium enriched yeast (0.30 mg/kg Se as Se-yeast) or selenium enriched yeast (0.30 mg/kg and 0.45 mg/kg Se as Se-yeast). Growth performance, feed to gain ratio, carcass and meat quality (pH, drip and cooking losses, colour, GSH-Px activity and chemical analysis) did not show any difference between the treatments. Meat colour and oxidative stability during 9 days of refrigerated storage were unaffected by dietary supplementation, suggesting that, at the levels of Se used in this experiment, dietary Se, even from an organic source, had limited potential for reducing lipid oxidation. Selenium supplementation raised the Se content in muscle (P < 0.001) with the greatest increase when Se-yeast was fed. Although selenite increased total Se, it did not influence total or specific selenoamino-acids in this tissue. On the contrary, Se-yeast supplementation led to an increase in muscle Se-methionine content. It was concluded that Se supplementation can increase significantly
muscle Se levels and produce, particularly when Se-yeast is fed, a source of Se enriched meat as Se-methionine.

Juniper et al. (2009a) examined the effect of feeding stuff supplementation with selenium-enriched yeast and sodium selenite on the quality of lamb meat. Total selenium content, selenomethionine and selenocysteine content and oxidative stability of meat after slaughtering were examined due to consumption of selenium-enriched yeast and sodium selenite. During the 112-day-experiment done with 50 lambs, the feeding stuff was supplemented with different amount of selenium-yeast and sodium selenite. At the beginning of the experiment and at day 28, 56, 84 and 112 blood sample was taken and the amount of selenium as well as various selenium-containing compounds and glutathione peroxidase activity were measured. At the end of the experiment the animals were slaughtered, then determination of selenium and various selenium species were carried out in heart, liver, kidney and skeletal muscle. As the lambs received feedstuff supplemented with selenium yeast total selenium level, selenomethionine level of the blood and glutathione peroxidase activity of the erythrocytes increased, however, no change was experienced during the supplementation with sodium selenite. With the exception of kidney tissue, all the other tissues showed a dose-dependent change regarding both total selenium content and selenomethionine content during supplementation with selenium-containing yeast. Based on a longer term examination, selenium content was higher for animals fed with selenium yeast than for those that were fed with sodium selenite, which means a better utilization for selenium from the yeast. Selenium or selenocysteine supplementation did not affect the glutathione peroxidase activity and thiobarbituric acid reactive substances. Despite this, the oxidative stability was slightly higher due to selenium supplementation.

Juniper et al. (2009b) examined the effect of high-dosage selenium yeast supplementation on selenium content of lamb issues and on different selenium compounds. 32 lambs weighing 6.87 kg were fed with milk-replacing feed preparation supplemented with 6.3 mg/kg selenium on dry-matter basis. Selenium content of feed of the control lambs that received no selenium supplementation was 0.13 mg/kg. The experiment was carried out during 91 days, and blood samples were taken on days 28, 56 and 91. At the end of each treatment five lambs were slaughtered and selenium content of the heart, liver and skeletal muscles was examined. Total selenium content of blood of group treated with selenium yeast was 815 ng/ml, this value for the control group was 217.8 ng/ml. Total selenium content of the tissues in the groups received selenium supplementation was significantly higher compared to the
control group (26 times higher in the skeletal muscle, 16 times higher in the liver, 8 times higher in the heart and 3 times higher in the kidney). Total selenium content as well as selenomethionine and selenocysteine content differed considerably in the individual tissues. Selenocysteine was the dominant amino acid in the liver and kidney. It was established that due to the selenium supplementation total selenium content of tissues increased and also that total amount of selenium and selenoamino acid was different due to the treatments.

Kumar et al. (2009) studied the effect of inorganic and organic selenium supplementation on 18 lambs of weight of 24.68 kg and aged 8-9 months. The lambs were divided randomly into six different groups. Feeding stuff of the control group consisted of maize groats, soybean flour, wheat bran, mineral supplementation without selenium, common kitchen salt and wheat straw. Beyond this, the experimental group received 15 mg/kg selenium in the form of sodium selenite, the other the same quantity but in the form of organic selenium supplementation. The experiment was conducted 90 days long. In order to trigger the humoral immune response the animals were vaccinated with Haemorrhagic septicaemia vaccine, and at the beginning of the experiment, on day 30, 60 and 90 blood sample was taken. Selenium supplementation had no effect whatsoever on the total cholesterol, total protein, albumin and globulin amount of the serum, the ratio of albumin and globulin, Ca and P level of the serum as well as activity of the measured enzymes (glutamate-oxalacetate-trans-amilase, glutamate-pyruvate-trans-amilase). In opposition to it, compared to the control group selenium level of the serum significantly increased as well as the number of the red blood cells and the humoral immune response, for both groups received selenium supplementation. The daily weight gain was the highest for the group received organic selenium supplementation, followed by the group received inorganic selenium supplementation, and the lowest value was measured for the control groups. The supplementation with the organic and inorganic selenium improved the growth indices. Regarding the humoral immune response and antioxidant status of the lambs the organic selenium proved to be more effective.

Hall et al. (2009) examined selenium status of sheeps due to short term high selenium feeding and mineral supplementation, respectively. Sheep grazed in Se-deficient areas without access to Se supplements may be Se deficient by the end of the grazing season. One limitation to feeding mineral mixes and feeds containing inorganic Se-supplements is the short duration of Se storage in the animal. Another is that Se supplementation may be limited by country-specific regulations. However, the use of feedstuffs naturally high in Se to deliver supranutritional levels of Se is not regulated. The purpose of this
study was to evaluate Se status in sheep after short-term exposure to high-Se-fertilized forage vs. a commonly used mineral supplement containing inorganic sodium selenite. Selcote Ultra® was mixed with nitrogen fertilizer in the form of urea and applied to pasture at a rate of 3.4 kg Selcote Ultra®/ha and 67.4 kg nitrogen/ha. Thirty ewes were randomly divided into two groups. One group of 15 ewes grazed Se-fertilized pasture for 40 days and had no mineral supplementation. The other group of 15 ewes grazed a non-Se-fertilized pasture and received a custom made mineral supplement containing 200 mg/kg sodium selenite for 40 days. Subsequently the two groups of ewes were combined and grazed a non-Se-fertilized pasture and had no mineral supplementation. Sheep were bled pre- and post-treatment and then every 2-4 weeks thereafter for approximately 9 months to assess whole-blood Se concentrations. Whole-blood Se concentrations were higher \((P < 0.0001)\) immediately post-treatment in sheep grazing Se-fertilized forage \((573 \pm 20 \text{ ng/ml})\) compared to sheep receiving mineral supplement containing Se \((286 \pm 20 \text{ ng/ml})\), and were still higher \((P < 0.0001)\) at the end of 9 months \(97 \pm 7 \text{ ng/ml} \) vs. \(61 \pm 7 \text{ ng/ml} \) respectively. Whole-blood Se concentrations were within the normal reference interval \((150-500 \text{ ng/ml})\) for a longer period of time in sheep grazing Se-fertilized forage \((7 \text{ months})\) compared to sheep receiving mineral supplement containing inorganic Se \((4 \text{ months})\). No sheep showed clinical signs of ill-effects from Se supplementation. In conclusion, short-term exposure of sheep to Se-fertilized forage results in whole-blood Se concentrations sufficient to maintain adequate concentrations throughout grazing periods when there is limited access to Se supplements.

The role of selenium in the human nutrition – consequences of selenium deficiency

In humans two diseases have been associated with severe selenium deficiency: the Keshan disease (endemic cardionyopathy) and Kaschin-Beck disease (an osteoarthropathy). Keshan disease is endemic in children and in women in childbearing age, and occurs from north-east to south-west China. These areas are characterised by very low selenium availability in soil and extremely low selenium concentrations in crops \((Combs, 2001; FAO, WHO, 2001; Tan and Huang, 1991)\).

According to Boldery et al. (2006) lack of vitamins and minerals was brought into connection with cardionyopathic diseases already in the 30’s of the last century. (Cardionyopathy (CMP) is a collective term for heart diseases leading to weakening of pump functions of the heart, attributed to the own disease
of the muscle.) Dilatative cardiomyopathic diseases caused by selenium deficiency were first reported in 1935 in China’s Keshan county. In their publication a cardiomyopathic disease is reported that can cause also loss of weight and problems of pancreas. The patient who was treated for heart complaints, lost weight after surgical treatment. After he received selenium supplementation his health condition and heart functions considerably improved. This case draws the attention that selenium deficiency can be a cause of cardio-vascular diseases.

Keshan disease is caused probably not by selenium deficiency alone but also infection with a coxsackie virus which is initially not virulent but after being in contact with a selenium deficient patient, becomes active (Beck et al., 2004; FAO, WHO, 2001). The Kaschin-Beck disease was detected in China in children aged 5-13 years, and less extensively in south-east Siberia (FAO, WHO, 2001). Apart from selenium deficiency this disease can be associated with mycotoxins in foods and fulvic acids in drinking water.

It appears to be evident that even a less apparent selenium deficiency can affect human health in various ways because it affects the immune functions, viral infections, male fertility, thyroid function, asthma and inflammatory diseases (Rayman, 2000, 2002). Selenium may play a role in the prevention of cardiovascular diseases, however, this has not been proved to be unequivocal (Rayman, 2000, Stranges et al., 2006).

Increasing number of evidence has shown anticarcinogenic effect of selenium (Combs, 2005; Combs et al., 2001; Whanger, 2004). Epidemiological studies showed a significant negative correlation between cancer mortality rates and selenium content of forage crops in some US counties (Clark et al., 1991). Several clinical trials with humans have shown beneficial effect of selenium on reduction of cancer (Combs, 2005; Whanger, 2004). In an experiment the participants received a daily dose of 200 µg of selenium as selenium-enriched yeast it was found that selenium supplementation had no significant effect on non-melanoma skin cancer, but it reduced significantly the total cancer incidence, total cancer mortality and the incidences of prostate, colon and lung cancers (Clark et al., 1996, 1998). These clinical results are consistent with studies on small animals where selenium was shown to have antitumour effect (Whanger, 2004).

Hartikainen (2005) investigated biogeochemistry of selenium and its impact on food chain quality and human health. In areas where soils are low in bioavailable selenium (Se), potential Se deficiencies cause health risks for humans. Though higher plants have been considered not to require this element, the experience with low-Se soils in Finland has provided evidence that the
supplementation of commercial fertilizers with sodium selenate affects positively not only the nutritive value of the whole food chain from soil to plants, animals and humans but also the quantity of plant yields. The level of Se addition has been optimal, and no abnormally high concentrations in plants or in foods of animal origin have been observed. Se levels in serum and human milk indicate that the average daily intake has been within limits considered to be safe and adequate. In fact, plants act as effective buffers, because their growth is reduced at high Se levels. They also tend to synthesize volatile compounds in order to reduce excess Se. On the other hand, when added at low concentrations, Se exerts a beneficial effect on plant growth via several mechanisms. As in humans and animals, Se strengthens the capacity of plants to counteract oxidative stress caused by oxygen radicals produced by internal metabolic or external factors. At proper levels it also delays some of the effects of senescence and may improve the utilization of short-wavelength light by plants. High additions are toxic and may trigger pro-oxidative reactions. Thus, the present supplementation of fertilizers with Se can be considered a very effective and readily controlled way to increase the average daily Se intake nationwide.

Selenium intake of humans ranges between very wide limits due to the consumption of foods with different selenium content. Combs (2001) showed in his study that in the different parts of the world there can be a difference of orders of magnitude in the selenium intake of people. In China for example in the Keshan area the selenium intake varies between 7-11 µg/day, whereas in the Enshi county of central China it can reach several thousands µg per day. In Europe selenium consumption of adults is around 30-100 µg per day, in North America 60-220 µg/ and in New Zealand’s selenium deficient areas in some populations the daily selenium intake ranges between 19-80 µg/day. In some European countries selenium intake has decreased significantly in recent decades (Rayman, 2002). For example, average selenium intake in UK for adults has decreased from 60-63 µg/day measured in the 1970s to 29-39 µg in 1995 (Ministry of Agriculture Fisheries and Food, 1997; Rayman, 2002). The main reason for this is the decreased import of breadmaking wheat from North America which contains generally much more selenium than the wheat grown in the UK.

It appears that there is no general agreement as regards the sufficient selenium intake for humans (Thomson, 2004). The minimum selenium intake for the prevention of the Keshan disease was found to be around 17 µg/day (Yang and Xia, 1995), however, selenium intake for the maximum plasma GPx activity is estimated to be about 45 µg/day (Thomson, 2004). In the United
In the United States and Canada the recommended daily dietary allowance is 55 μg/day. The European population reference intake is also set at 55 μg/day. In Australia and New Zealand the recommended dietary intakes for male and female adults are 70 and 60 μg/day, respectively. In the UK the reference nutrient intake is set at 75 μg and 60 μg/day for male and female adults, respectively. In contrast, the WHO and FAO normative requirement estimates are 40 μg and 30 μg/day for men and women, respectively. Based on the FAO and WHO norms it is not possible to reach the maximal GPx activity. Further selenium intake is recommended for e.g. cancer prevention (Rayman, 2002). Combs (2001) suggested that a plasma selenium level above 120 μg/l may be useful for minimizing the risk of cancer. To achieve this, the dietary selenium intake should be at least 1.5 μg/kg body weight per day which is equivalent to 90-120 μg/day for a 60-80 kg person. Further research is needed to determine the minimum selenium intakes for a protective effect. Based on surveys of plasma or serum selenium levels Combs (2001) estimated that worldwide 0.5 and 1 billion people may be selenium deficient. In many European countries current plasma or serum selenium concentrations are below the level required for the maximum activity of plasma GPx.

Rasmussen et al. (2009) examined in Denmark the change in serum total selenium and selenomethionine content during eight years with special respect to the effect associated with selenium status. Blood samples were taken from 817 randomly chosen people, and and information on smoking habits, alcohol consumption and sporting activity was collected by questionnaire. For men the average serum selenium level was 98.7 μg/l, the selenoprotein level was 2.72 mg/l. Both selenium and selenoprotein level of the serum increased with the age and the selenoprotein level was higher for men than for women. Selenium level of the serum decreased by around 5% between 1997-2005, at the same time the selenoprotein level significantly increased. Fish consumption had only a very slight effect on the selenium level and did not influence the selenoprotein level at all. Smoking, alcohol consumption, physical exercises or drug consumption did not affect selenium status. It was found that selenium status of the Danish population was appropriate. No groups could be found where according to age, sex and lifestyle special attention should be paid to the selenium deficiency.

Toxicity of selenium

Excessive selenium intakes can lead to chronic toxicity (selenosis) with health damages as loss of hair and nails, skin lesions, hepatomegaly, polyneuritis
and gastrointestinal disturbances. Chronic selenosis was reported from Enshi county in central China where soil, locally produced foods, and water contain extremely high levels of selenium (Combs, 2001; Yang and Xia, 1995). Studies in this county showed that the toxic dietary selenium intake that would maintain the characteristic selenosis symptoms (fingernail changes) was approximately 1600 µg/day (Yang and Xia, 1995). A reduction of the selenium intake to 819 ± 126 µg/day enabled five selenosis patients to recover from fingernail lesions. Based on these studies Yang and Xia (1995) suggested that 600 µg/day was the individual daily maximum safe intake and recommended a maximum dietary selenium intake of 400 µg/day.

2 Selenium content in wheat and bioavailability of the selenium content

Contribution of the cereals to the selenium need of humans

Cereals, meats and fish are the main sources of selenium in the human diets (Combs, 2001). Cereals and cereal products contribute around 70% to the total dietary selenium intake in the low selenium areas of China, and 40-50% in the low-income population in India. In the UK a survey carried out in 1995 estimated that cereals and cereal products accounted for 18-24% of the total selenium intake. A survey in 27 regions of Russia showed a highly significant correlation between serum selenium and selenium content in wheat flour which indicates that wheat is an important source of selenium for the Russian population (Golubkina and Alfthan, 1999).

Selenium content of wheat grown on different soils

Cereals and cereal products contain a wide range of selenium concentration, most being between 10-550 µg/kg on fresh weight basis (FAO, WHO, 2001). More extreme values have also been reported, e.g. cereal grains produced in the Keshan county in China contain 3-7 µg/kg selenium, whereas wheat grain produced in North and South Dakota in the US may contain more than 2000 µg Se/kg. The nutritional minimum level both for animals and humans is about 50-100 µg Se/kg in dry fodder/food, and intake below that may cause selenium deficiency (Gissel-Nielsen et al., 1984).

Based on previous studies in China, Tan (1989) proposed the following ranges of grain selenium concentrations: below 25 µg/kg deficient, 25-40 µg/kg marginal, 40-1000 µg/kg moderate to high, and above 1000 µg/kg excessive.
In general, European wheats contain lower levels of selenium than North American wheats. Low selenium concentrations have been reported in Scandinavian countries with concentration in wheat ranging between 7-18 µg/kg (Gissel-Nielsen et al., 1984).

Murphy and Casman (2001) examined selenium content of foods consumed in Ireland. In the last 20 years in the UK and in other countries in Europe the selenium intake decreased due to the reduced importation of wheat with high selenium and protein content from North America and Canada. There are no results about selenium content of the Irish flour, bread and other foods, therefore it is difficult to estimate the daily selenium intake in Ireland. For this reason selenium content of various Irish foods, especially bread and flour were measured, after acidic digestion by hydride generation atomic absorption spectrophotometry. Less fine wheat flour had a higher selenium content (7.7-9.9 µg/100 g) than the finest wheat flour with a selenium content ranging between 6.0-6.9 µg/100 g. Selenium content of the Irish brown bread (8.6-12.9 µg/100 g) was higher than that of the white bread (6.6 µg/100 g). It was found that the Irish flours and breads did not contain so much selenium as those from North America or Canada, and contained only a little more selenium that those currently being consumed in the UK.

British wheat also has low selenium status (Barclay and Macpherson, 1986). Adams et al. (2002) conducted a survey of 452 grain samples of breadmaking wheat produced in the UK in the 1982, 1992 and 1998 seasons and reported a range of 6-858 µg Se/kg dry weight with mean and median values of 32 and 22 µg/kg, respectively. On a fresh weight basis and assuming a 15% moisture content, the mean and median values were 27 and 18 µg Se/kg, respectively. 91% of the samples contained less than 50 µg Se/kg fresh weight. In comparison, Wolnik et al. (1983) reported a range of 10-5300 µg Se/kg fresh weight with mean and median values of 160 and 370 µg Se/kg, respectively, for 290 wheat samples collected from major growing areas in US. Wheat samples from Manitoba of Canada had a mean selenium concentration of 760 µg/kg (Boila et al., 1993). These surveys indicate that North American wheats contain on average more than 10-fold larger concentrations of selenium than British wheats. Similarly, selenium concentrations in wheats produced in New Zealand are considerably lower than those produced in Australia, and the importation of Australian wheat was found to be an important beneficial factor affecting the blood selenium status in the residents of Hamilton area of New Zealand (Watkinson, 1981). A recent surveys of South Australian wheats reported a range of 5-720 µg Se/kg, with a mean value of 155 µg/kg (Lyons et al., 2005b).
Lyons et al. (2005a) examined selenium status of wheat in Australia and found that wheat (Triticum aestivum L.) contributed to the greatest extent to the average plasma selenium concentration of 103 μg/l. By the analysis of selenium content of 834 blood plasma in six experiments was obtained that the selenium content was higher in males and increased with the age. This study showed that many South Australians consume inadequate amount of selenium to maximise selenoenzyme expression and cancer protection, and indicated that levels declined around 20% from the 1970’s. No significant genotypic variability for grain selenium concentration was observed in modern wheat cultivars, but the diploid wheat was higher. Grain selenium concentration ranged between 5-720 μg/kg, and it was apparent that this variation was determined mostly by available selenium content of the soil. In both glasshouse and field trials selenium applied as sodium selenate at rates of 4-120 g Se/ha increased grain selenium concentration progressively up to 133-fold value when sprayed on soil at seeding and up to 20-fold when applied as a foliar spray after flowering. A threshold of toxicity of around 325 mg Se/kg in leaves of young wheat plants was observed, a level that would not be normally reached with selenium fertilisation. On the other hand sulphur applied at the low rate of 30 kg/ha at seeding reduced grain selenium concentration by 16%. It was established that agronomic biofortification could be used by food companies as a cost-effective method to produce high-selenium wheat products that contain most selenium in the desirable selenomethionine form. Further studies are needed to assess the functionality of high-selenium wheat, e.g. short-term clinical trials that measure changes in genome stability, lipid peroxidation and immunocompetence. Increasing selenium content of wheat is a food system strategy that could increase the selenium intake of whole population.

Lyons et al. (2005b) analyzed selenium content of various wheats grown in Mexico and Australia and other commercial cultivars. Cultivars were also grown under the same conditions to assess genotypic variation in selenium density. Selenium content of the grains varied between 5-720 μg Se/kg and this variation could be associated with the selenium content of the soil. On identical soils no significant genotypic variation could be found among modern commercial bread and durum wheat, triticale or barley. However, the diploid wheat and rye were 42% and 35%, respectively, higher in grain selenium concentration than other cereals in separate field trials, and in a hydroponic trial rye was 40% higher in foliar selenium content than two wheat landraces. While genotypic differences may exist in modern wheat varieties, they are likely to be small in comparison with background soil variation, at least in South Australia and Mexico.
Effect of the technology (milling) on the selenium content of flour

Milling of wheat has only a small effect on the selenium concentration of flour fractions. A study by Eurola et al. (1991) showed that wheat bran and flour fractions contained slightly more selenium than other flours, whereas selenium levels of breads were somewhat lower than those of the corresponding flours. Similarly, Lyons et al. (2005a) found selenium to be fairly evenly distributed throughout in the wheat grain, except that the embryo tended to have a higher concentration than the other milled fractions. It was established that further processing did not affect selenium content of wheat products.

Selenium forms in foodstuffs and in wheat

Selenium exists in foods in different chemical forms. Guo and Wu (1998) examined the free selenoamino acids in the plant tissues and their distribution in high-selenium soils. Accumulation of the selenoamino acids in the vegetable tissues is associated with not only with the tolerance against selenium of the plants but also selenium poisoning of animals. Selenoamino acid content of the selenium-tolerant plants was examined by high-resolution gas chromatography coupled with mass spectrometer. Five selenoamino acids (selenocystine, selenomethionine, selenocysteine, Se-methyl-selenocysteine and γ-glutamyl- Se-methyl-selenocysteine) were identified in the vegetable tissue concentrates. The amount of Se-methyl-selenocysteine was 15.3 μmol/kg in the plants grown on low-selenium soils and 109.8 μmol/kg in the plants grown on high-selenium soils. Also γ-glutamyl-Se-methyl-selenocysteine was detected although in a very low concentration. Selenium-accumulation experiments showed that concentration of selenocysteine increased to 5-fold in the vegetal tissues, while the total amount of selenium ranged from 5.07 to 22.02 mg/kg, but no further increase in the selenocysteine concentration was found with the further increase of the selenium concentration from 22.0 mg/kg to 117.5 mg/kg. It was found that selenomethionine in the plants represented more than 50% of total selenium. Further investigations are necessary in order to find out what mechanism affects the accumulation of selenocysteine in plants.

A range of selenocompounds have been identified in plants such as selenate, selenite, selenocysteine (Se-Cys), selenomethionine (Se-Met), selenohomocysteine, Se-methyl-selenocysteine (MeSeCys), γ-glutamyl-Se-methyl-selenocysteine, selenocystineselenenic acid, Se-propionylselenocysteine selenoxide, Se-methylselenomethionine (SeMM), seleno-cystathionine, dimethyl diselenide, selenosinigrin, selenopeptide and selenovax (Whanger, 2002).
Se-Met is the predominant form of Se in the wheat grain (56-83%), with other selenocompounds existing in smaller proportions: selenate (12-19%), Se-Cys (4-12%), Se-methyl-selenocysteine (1-4%), and others 4-26% (Whanger, 2002). In contrast, over 50% of the Se in wheat straw is selenate. An enzymatic hydrolysis released 70% of the Se in a wheat flour sample, of which Se-Met and Se-Cys accounted for 73% and 27% of the released selenium, respectively (Moreno et al., 2004). Stadlober et al. (2001) reported that the enzymatic hydrolysis of wheat, barley and rye flour samples released 80-95% of the total Se, with 62-86% being Se-Met.

3 Bioavailability of selenium content of different foods

The bioavailability of selenium varies between different foods. SeMet (in plant and animal sources) and SeCys (mainly in animal sources) have high bioavailability (more than 90%), whereas the bioavailability of the inorganic selenate and selenite exceeds 50% (Thomson, 2004). Selenium in wheat grain has high bioavailability. In a feeding trial with rats, wheat Se had a bioavailability of 83%, compared to mushrooms (5%), tuna (57%) and beef kidney (97%) (Thomson, 2004). A study in humans showed that the inclusion of Se-enriched wheat in the diet for six weeks increased serum selenium significantly, whereas the consumption of selenium-enriched fish gave no significant effect (Meltzer et al., 1993). Fox et al. (2005) compared the efficiency of selenium absorption in three food sources by humans using intrinsic labelling with the stable isotopes 77Se and 82Se. They found that Se-absorption was significantly higher from wheat (81%) and garlic (78%) compared to fish (56%). Due to the high bioavailability of selenium, wheat would be a good choice for biofortification in order to enhance selenium intake by humans.

Effect of selenium on the activity of the enzymes of wheat

Nowak et al. (2004) examined the effect of selenium on the activity of oxido-reductase enzymes in soil and plants. In glasshouse trials the effect of 0.015; 0.15; 0.45 mmol/kg hydrogen selenide concentration was examined on the activity of the oxido-reductase enzymes of in wheat. Hydrogen selenide increased in each concentration the activity of nitrate reductase, but in plants the polyphenol oxidase activity was increased by the two lower concentrations only. The highest selenium concentration inhibited both the polyphenol
oxidase and peroxidase. In the plants the catalase activity reduced in the concentration range of 0.15-0.45 mmol/kg. It was found that the peroxidase activity in soil decreased due to the uptakable selenium content. The lowest selenium dose significantly (by 10%) increased the catalase activity, however, the effect of the higher doses on the enzyme activity is uncertain.

Bioavailability of selenium in soils to plants

If no direct selenium supplementation is applied, the selenium status in humans is determined by the supply of selenium in the soil to plants, which is governed largely by the underlying geology. The Earth’s crust has an average selenium concentration of about 0.05 mg/kg (McNeal and Balistrieri, 1989). Magmatic rocks generally contain less selenium than sedimentary rocks especially shales (Mayland et al., 1989). The concentration of selenium in most soils lies within the range of 0.01-2 mg/kg (Kabata-Pendias and Pendias, 1992). Selenium content of the soil in some parts of the world is low, including Nordic European countries, New Zealand, eastern and central Siberia, and the Keshan disease belt in China. These areas are notable also for having low selenium status in forage and food crops, humans and animals. Studies in New Zealand showed a high incidence of selenium-responsive disease in sheep in areas with soils containing less than 0.5 mg Se/kg (Oldfield, 1999). Tan (1989) defined the Se status for human nutrition according to the concentration of total selenium in soil as: deficient if less than 0.125 mg/kg; marginal 0.125-0.175 mg/kg; 0.175-3 mg/kg moderate-high; and excessive if above 3 mg/kg, respectively. Soils in England and Scotland tend to have relatively low selenium contents (Oldfield, 1999). A geochemical survey of the UK showed a range of total selenium in soils from 0.1 to 4 mg/kg, with more than 95% of the samples containing less than 1 mg/kg (Broadley et al., 2006).

In contrast to the low Se soils, some areas in the world (e.g. the Great Plains of the US and Canada, Enshi county of China, parts of Ireland, Colombia and Venezuela) are seleniferous (Combs, 2001), with the soils having developed mainly from Se-enriched shales (Mayland et al., 1989). Seleniferous soils are generally defined as those bearing vegetation containing more than 5 mg Se/kg, and are associated with Se poisoning of livestock and wild life (Gupta and Gupta, 1998; Oldfield, 1999). The total Se concentrations in seleniferous soils are usually in the range of 5-1200 mg/kg (Mayland et al., 1989). Selenium deficient and selenium-toxic environments have been shown to occur within 20 km of each other in the Enshi county of China as a result of the variation in the underlying geology (Fordyce et al., 2000a).
Soil conditions such as pH, soil texture and the contents of iron oxide/hydroxides and organic matter have a significant influence on the bioavailability of Se to plant uptake (Gissel-Nielsen et al., 1984; Mikkelsen et al., 1989). Soil pH and Eh affect the chemical species of Se present in soil (Elrashidi et al., 1987). Selenate is the predominant form in alkaline and well-oxidised soils, whereas in well-drained mineral soils with pH from acidic to neutral selenium exists predominantly as selenite. Under strongly reduced soil conditions selenide becomes the dominant form. Selenite is much more strongly adsorbed by the adsorbing surfaces of soils than selenate, and the adsorption of both decreases markedly with increasing pH (Barrow and Whelan, 1989). Selenate is only weakly adsorbed through a non-specific mechanism based on electrostatic forces, similar to the adsorption of sulphate, whereas the mechanism of selenite adsorption appears to be an innersphere surface complexation, similar to that for phosphate adsorption (Barrow and Whelan, 1989; Neal et al., 1987). Neal and Sposito (1989) found no adsorption of selenate in alluvial soils from California over the pH range of 5.5-9. This means that selenate is more soluble and mobile than selenite in soil, and is therefore more bioavailable to plants but also more prone to leaching. Selenium bioavailability to plants generally decreases with decreasing pH and with increasing contents of clay, iron oxides and hydroxides and organic matter (Gissel-Nielsen et al., 1984; Johansson, 1991; Mikkelsen et al., 1989). High contents of iron oxides and hydroxides and of organic matter, and low pH in soil have been identified as important factors contributing to the incidence of Keshan disease in China (FAO, WHO, 2001; Fordyce et al., 2000b; Johnson et al., 2000). Soil compaction and irrigation influence selenium concentration in wheat grain. Irrigation resulted in a 10-fold decrease in selenium concentration, possibly due to increased leaching losses of selenium, an antagonistic effect of sulphur in the irrigation water and a dilution effect from a higher grain yield (Zhao et al., 2007). Soil compaction also led to a significant reduction in grain selenium concentration.

Zhao et al. (2006) investigated the effect of soil compaction and irrigation on grain selenium concentration. Grain selenium concentration ranged between 10-115 μg/kg which decreased by 30-75% due to irrigation. Significant selenium reducing effect of soil compaction has been explained by different mobility of the element due to different ionic transport from soil to the root. The observed effects in grain selenium content are considerable both in the respect of human nutrition and animal feeding because the concentration can vary from the sufficient to the very low level. It was found that the physical condition of the soil was always to be considered when the bioavailability of selenium is assessed.
Fan et al. (2008) examined the change in selenium content of soil and wheat grain in the last 160 years in the UK where the daily selenium consumption has been considerably decreased since the 1970’s. In order to decide whether this was caused by the change in wheat grain selenium concentration or by a change in the environment, cereal grains, wheat grains and soil samples taken from areas differently fertilized were retrospectively analyzed back in the past 160 years. Grain selenium content ranged between 11 and 236 ng/g. Grain samples collected from non-fertilized areas contained significantly more selenium than those from fertilized soils. No significant difference was found when short-straw wheat samples from the 1960’s were analyzed. Wheat samples before 1920 and after 1970, respectively, contained more selenium than those between 1920 and 1970, although soil selenium content increased continuously during the passed 160 years. Based on the obtained results it was established that wheat grain selenium content affected by the sulphur emission into the atmosphere and the transfer from the atmosphere into the soil, respectively, but the yield increased by the plant breeding did not reduce significantly selenium content in the fertilized samples.

Zhao et al. (2009) examined selenium content of 150 bread wheat varieties cultivated at different places and in different seasons. No considerable difference was found in the selenium content among the expressly bread wheat varieties, which was chiefly affected by the type of soil. Selenium content was not dependent on which part of the wheat grain was sampled.

Possibilities of increasing selenium content of foods by increasing selenium content of the soil

Selenium content in food and forage crops can be increased by the addition of selenium to the soil-crop systems, a practice termed agronomic biofortification. The best example is the Finnish practice of adding sodium selenate to all multi-element fertilizers, which has occurred since 1984 (Eurola et al., 1991; Hartikainen, 2005; Yläranta, 1990). Initially, 6 mg Se/kg fertilizer for grass and hay crops and 16 mg Se/kg fertilizer for cereals were used. These levels of addition provided approx. 3 and 8 g Se/ha for grass and cereals, respectively (Eurola et al., 1990). Selenium concentrations in plants, animal products, soils, water and human sera have been monitored regularly, and the results have been used to adjust the amount of selenium addition. Between 1991-1997 the lower level (6 mg Se/kg fertilizer) was used for all crops. Since 1998 the selenium concentration has been raised to 10 mg Se/kg fertilizer (Hartikainen, 2005). This practice has substantially increased Se concentra-
tions in crops, vegetables, fruits and animal products. For example, the mean Se concentrations in all Finnish cereal grains were below 10 µg/kg dry weight before Se fertilization, and were increased to 250 µg/kg for spring wheat, 50 µg/kg for winter wheat, 40 µg/kg for rye, 170 µg/kg for wheat flour and 180 µg/kg for wheat bread, respectively, in the first three growing seasons after Se fertilization (Eurola et al., 1990). As a result, the average Se intake in Finland increased from 25 µg/day before Se fertilization to around 110 µg/day (Eurola et al., 1991), and the serum Se level increased from 60-70 µg/l to more than 100 µg/l (Varo et al., 1988). The contribution of cereals to the total Se intakes also increased from 9% to 26% (Eurola et al., 1991). Compared to direct selenium supplementation, agronomic biofortification is considered to be advantageous in that inorganic Se is assimilated by plants into organic forms (e.g. SeMet) which are more bioavailable to humans. In addition, plants act as an effective buffer that can prevent accidental excessive Se intakes by human that may occur with direct supplementation (Hartikainen, 2005).

Selenium fertilization has also been practiced in other regions of the world, such as for pasture in New Zealand and crops in the Keshan disease area in China. There have been numerous reports of Se-enriched plant products obtained by Se fertilization, including Se-enriched broccoli, garlic, onions, potatoes, mushrooms and tea. Both pot and field studies have shown that the addition of selenate increases plant selenium concentrations much more effectively than the addition of selenite (Gissel-Nielsen et al., 1984; Shand et al., 1992; Singh, 1991; Cartes et al., 2005), therefore selenate is more widely used in Se fertilization and is available in a range of commercial Se fertilizers (Broadley et al., 2006). It is important that field experiments are conducted under different cropping systems and climatic conditions to obtain reliable information on the optimum rate of Se fertilization. Field studies in Canada showed that the addition of 10 g Se/ha was necessary to ensure adequate selenium concentration (above 100 µg/kg) in barley grain (Gupta, 1995). Tveitnes et al. (1996) applied calcium nitrate enriched with 25 mg Se/kg as a top dressing for spring wheat in a Norwegian field trial. This provided 6.5 g Se/ha and increased the Se concentration in wheat grain to the desired level. In general, there is a little residual value to subsequent crops from appropriate additions of Se applied to previous crops, suggesting that the applied selenium which is not taken up by the plants is either fixed in the soil or lost to the environment. Monitoring of the environment is also required to ensure that agronomic biofortification of selenium does not lead to significant enrichment in water bodies. Finnish data show little evidence of Se enrichment in lake ecosystems (Mäkelä et al., 1995; Wang et al., 1995), although some ground water samples showed simultaneous
increases in total N, P and Se concentrations that may have resulted from the leaching of selenium from the fertilizers into the ground water. (Mäkelä et al., 1995).

Lavado et al. (1999) examined the effect of agriculture and fertilization on extractable selenium content of soil. No difference was found due to fertilization and different agricultural technologies in case of soils with average selenium content of 3.33 mg Se/kg.

Martens and Suarez (1999) investigated the transformations of volatile methylated selenium in soil. Microbial volatilization of selenium as dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe) from soil is an important part of the Se cycle in nature, but little is known about the stability and transformations of these gases during residence in the soil environment before dissipation to the atmosphere. Experiments monitored by gas chromatography and atomic absorption spectroscopy were made with various clay mineral standards, charcoal, commercial humic substances and soils to determined the sorption and transformations of DMSe and DMDSe injected onto the headspace or passed through soil materials. Batch experiments conducted with 2-5 g materials placed into 40 ml Teflon centrifuge tubes equipped with Mininert gas sampling valves showed that DMSe was slowly sorbed by soil materials and most of the DMSe deficit in the headspace was recovered as selenite and selenate. In contrast, DMDSe was rapidly partitioned from the gas phase and resulted in an increased recovery of less soluble elemental and selenide forms. These results were confirmed during flow-through soil column studies with both little DMSe sorption and sorption of the majority of the DMDSe additions. Additions of selenomethionine to soil to produce DMSe and DMDSe in sealed flasks resulted in an increased partitioning of Se into inorganic when compared with a flow-through system designed to limit the contact of Se gases with soil. These results suggest that soil Se volatilization as DMSe and DMDSe results in Se loss to the atmosphere as DMSe with concomitant soil Se immobilization due to the instability of DMDSe.

Tan et al. (2002) examined the relation between soil selenium content and endemic diseases in China. In their study the geographical distribution of the total and water-soluble selenium content in topsoil (plough layer for cultivated soils, eluvial horizon for natural soils) was discussed and its relationship with some human health problems with China was evaluated. Topsoil samples, 354 in total, including 156 natural and 198 cultivated soils of 21 main soil types were collected. The total selenium concentration in soil samples was determined after derivatization with DAN (diaminonaphthalene) by spectrofluorometry. Water soluble Se concentration in soil was determined by the
same method after extraction with water in a soil to water ratio of 1:5. The geometric and arithmetic mean of total Se concentration was 0.173 mg/kg and 0.239 mg/kg, respectively, with the lowest value being 0.022 mg/kg and the highest one being 3.806 mg/kg. For the cultivated soils, the geometric mean of the total Se content was 0.188 mg/kg, and its arithmetic mean was 0.269 mg/kg, these values for the natural soils were 0.154 mg/kg and 0.206 mg/kg, respectively. Geometric mean of the water-soluble Se content of the soils was 4.0 µg/kg, the arithmetic mean was 6.4 µg/kg, the lowest value was 0.6 µg/kg and the highest one was 109.4 µg/kg. For the cultivated soils the average concentration of water-soluble Se was 4.3 µg/kg, similar to that of the natural soils (4.4 µg/kg geometric mean). Two sequences of the soil types, arranged separately in the concentration of total Se and water-soluble Se, are different and this demonstrates that the proportions of the two forms of selenium existing in various soils are different. The percentages of water-soluble Se to total Se in different types of soils varied from 1.07 to 6.69%. The laterite and other subtropic soil have relatively high water-soluble selenium contents because of their higher total selenium contents. A very significant correlation between total Se and water-soluble Se has been found in cultivated soil with a correlation coefficient of 0.58.

Dhillon et al. (2006) conducted greenhouse experiments to study the bioavailability of selenium to sorghum (Sorghum bicolor L.), maize (Zea mays L.) and berseem (Trifolium alexandrinum L.) fodders in a sandy loam soil modified with different levels of Se-rich wheat (Triticum aestivum L.) and raya (Brassica juncea L. Czern) straw containing 53.3 and 136.7 µg Se/g, respectively. Application of Se-rich straws to each crop, even at the highest rate of 1%, did not have any detrimental effect on dry-matter yield of the different crops. With an increase in the level of wheat straw from 0% to 1%, Se content in sorghum and maize plants increased to the highest level of 1.3 and 1.5 µg/g, respectively, at 0.3% of the applied straw and thereafter it decreased consistently. In case of raya straw, the highest Se content in sorghum (2.3 µg/g) and maize (3.0 µg/g) was recorded at 0.3% and 0.4% of the applied straw, respectively. These investigations suggest that Se-rich raya and wheat straw may be disposed off safely in soils for growing fodders.

Hawkesford and Zhao (2007) analyzed various strategies for increasing the selenium content of wheat. Selenium is essential for humans and animals but has no known function in plants. Excess accumulation is toxic to both plants and animals. Dietary intake of selenium is low in a large number of people worldwide. This is due to low bioavailability of Se in some soils and consequently low concentrations of Se in plant tissues. Both selenate and selenite are
taken up by plants and subsequently translocated around the plant. Selenate is an analogue of sulphate and is transported by the sulphate transporter family. Some plants are able to accumulate high concentrations of selenium (hyperaccumulators), however, genetic variation in accumulation ability amongst non-accumulators such as cereals, is relatively small. Within plant tissues, selenium enters the pathways for sulphate assimilation and metabolism and will replace cysteine and methionine in proteins, often with detrimental effect. Alternatively, selenium may be accumulated as methylated derivatives or lost from the plant following volatilisation. Agronomic biofortification of crops with Se-containing fertilisers, which is practised in some countries, provides the best short-term solution for improving selenium content of wheat. Longer-term genetic improvement, particularly by targeting substrate discrimination of transporters between selenate and sulphate, for example, may provide a means to enhance uptake and promote accumulation.

Zhao and McGrath (2009) investigated soil selenium content and its reduction in experiments with plants. Plants and the associated rhizosphere microbes may be used to take up and/or volatilize excessive build-up of Se in contaminated soil and irrigation drainage water. Selenium, when present as selenate, is highly bioavailable to plant roots. Recent field trials have shown that transgenic Brassica Juncea (Indian mustard) overexpressing genes involved in sulphur/selenium metabolism have enhanced selenium accumulation and tolerance. The transgenic plants overexpressing adenosine triphosphate sulfurylase (APS), which catalyzes sulfate/selenate activation before they can be reduced to sulfite/selenite, accumulated 4.3-fold more Se than the wild-type plants, and extracted approximately 4% of the extractable Se from a contaminated soil. The major mechanism of Se toxicity in plants is the non-specific incorporation of selenocysteine and selenomethionine into proteins in place of Cys and Met, resulting in the alteration of protein structure. One way to enhance Se tolerance is to direct the metabolic flow of SeCys away from protein synthesis by overexpressing SeCys lyase (SL) which decomposes SeCys to elemental Se and alanine. Another way to engineer Se tolerance is to transfer the selenocysteine methyltransferase (SMT) gene from the Se hyperaccumulator Astragalus bisulcatus, which is also hypertolerant to Se, to nontolerant plants. SMT catalyzes the methylation of SeCys to methylselenocysteine, which is a non-protein amino acid non-toxic to plants. The SMT transgenic plants of B. Juncea accumulated 60% more Se from a contaminated soil than the wild-type under field conditions.

Darcheville et al. (2008) investigated the role of microorganisms in the behaviour of selenium in natural soils maintained under strictly aerobic con-
ditions. Six-day batch experiments were performed with soils constrained to different microbiological states, either by sterilization or by adding organic substrate. Selenium was added to the soil as selenite. The distribution of selenium in the gaseous, liquid and solid phases of the batch was measured. It was found that the active microorganisms played major role in the distribution of selenium within the soil. On the one hand microorganisms could promote volatilisation of selenium (in relatively small amounts), leading to the spreading of selenium compounds outside the soil. On the other hand microbial activity increased both amount of selenium retained by the soil, and the strength of its retention (less exchangeable selenium), making selenium less susceptible to remobilisation.

**Selenium uptake into wheat**

Increasing selenium content of wheat requires investigation of uptake mechanism catalyzed by transporters. Both selenite and selenate in the soil may be available to plants depending upon soil conditions. Several studies have demonstrated the uptake of selenite into plants (*Hopper and Parker*, 1999; *Zhang et al.*, 2003), although in some cases root to shoot translocation seemed to be more limited than for selenate (*Hopper and Parker*, 1999). Competition was demonstrated between selenite and phosphate in nutrient solution studies indicating a possible involvement of phosphate transporters in selenite uptake (*Hopper and Parker*, 1999), although this has not been investigated at the molecular level. It is generally accepted that selenate is taken up by plants from the soil via sulphate transporters in the roots. At the whole plant or agronomic scale the interaction between sulphate and selenate is well documented (*Barak and Goldman*, 1997; *Bell et al.*, 1992; *Broadley et al.*, 2006; *Hopper and Parker*, 1999; *Mikkelsen and Wan*, 1990; *Wu and Huang*, 1992). An implication of these interactions is that sulphate fertilization and selenium biofortification may be inextricably linked and are likely to be antagonistic to one another. The variation in the selectivity of the sulphate transporters for sulphate compared to selenate offers an opportunity for selective enrichment. Early kinetic studies of sulphate uptake into barley roots showed that selenate was a competitive inhibitor of sulphate uptake (*Legget and Epstein*, 1956). Based on this evidence antagonism for uptake is inevitable.
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References


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